American Journal of Hematology 52:254–257 (1996)

Vincristine-Induced Alterations in Schwann Cells of Mouse Peripheral Nerve

Ruth Djaldetti, Jacob Hart, Svetlana Alexandrova, Sergin Cohen, Ben-Zion Beilin, Meir Djaldetti, and Hanna Bessler

Department of Neurology (R.D.), Beilinson Medical Center, Petah Tiqva, and Department of Administration (J.H.), Hematology Research and Electron Microscopy Unit (S.A., M.D., H.B.), and Department of Anesthesia (B.B.), Hasharon Hospital, Golda Medical Center, Petah Tiqva, and Sackler Faculty of Medicine, Tel Aviv University, Israel

The sciatic nerve of C57Bl mice was examined with a transmission electron microscope to study the ultrastructural alterations in Schwann cells following treatment with escalating doses of vincristine. Results indicated that the drug exerts a dose-related effect. Total doses up to 8 μg/mouse did not cause any visible damage to Schwann cells. Higher doses induced not only damage to individual cells, but also affected a greater percentage of them. The myelin sheath was the most affected organelle. Schwann cells of myelinated fibers showed greater damage than those of unmyelinated fibers. © 1996 Wiley-Liss, Inc.

Key words: vincristine, Schwann cells, peripheral neuropathy, transmission electron microscopy

INTRODUCTION

Peripheral neuropathy is a well-documented complication of Vinca alkaloid administration, particularly vincristine (VCR) [1,2]. It is dose-related, and involves both the central and peripheral nervous system [3]. The peripheral neuropathy is most probably due to axonal damage consequent to disruption of the microtubules [2,4,5], although it has been suggested that this is not the only cause [3]. According to Neundorfer [6], the motor neurons are the most affected by VCR. Depression of the Achilles tendon reflex is an early and prominent sign of peripheral neuropathy in patients treated with VCR [2].

The aim of the present study was to examine the effect of escalating doses of VCR on the ultrastructure of the Schwann cells of the mouse sciatic nerve.

MATERIALS AND METHODS

Five groups of five C57Bl mice were used throughout the study. The first group was injected intravenously with saline for 12 days and served as control. Mice in the remaining four groups were treated intravenously with VCR (Pharmachie, Harlem, The Netherlands), as follows: group II, 2 μg/mouse for 4 days; group III, 2 μg/mouse for 12 days; group IV, 12 μg/mouse for 8 days; and group V, 100 μg/mouse for 4 days. The total amount of VCR per each mouse of the four groups was 8, 24, 96, and 400 μg, respectively.

Blood counts were taken before and at the end of the study. Following the last injection, the animals were anesthetized with ether, and the sciatic nerve was exposed, cut into small pieces, and transferred to cold 1% glutaraldehyde in phosphate buffer, pH 7.4. The specimens were postfixed in osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon 812 (Polaron Equip., Ltd., Watford, England). Thin sections, stained with uranyl acetate and lead citrate, were examined with a Philips 300 transmission electron microscope at an acceleration voltage of 60 kV. In addition to whole Schwann cells, 400 rings of myelin sheaths alone were counted, and the percentage of the damaged sheaths was detected.

Received for publication October 27, 1995; accepted February 7, 1996.

Address reprint requests to Prof. M. Djaldetti, Electron Microscopy Unit, Hasharon Hospital, Petah Tiqva 49372, Israel.

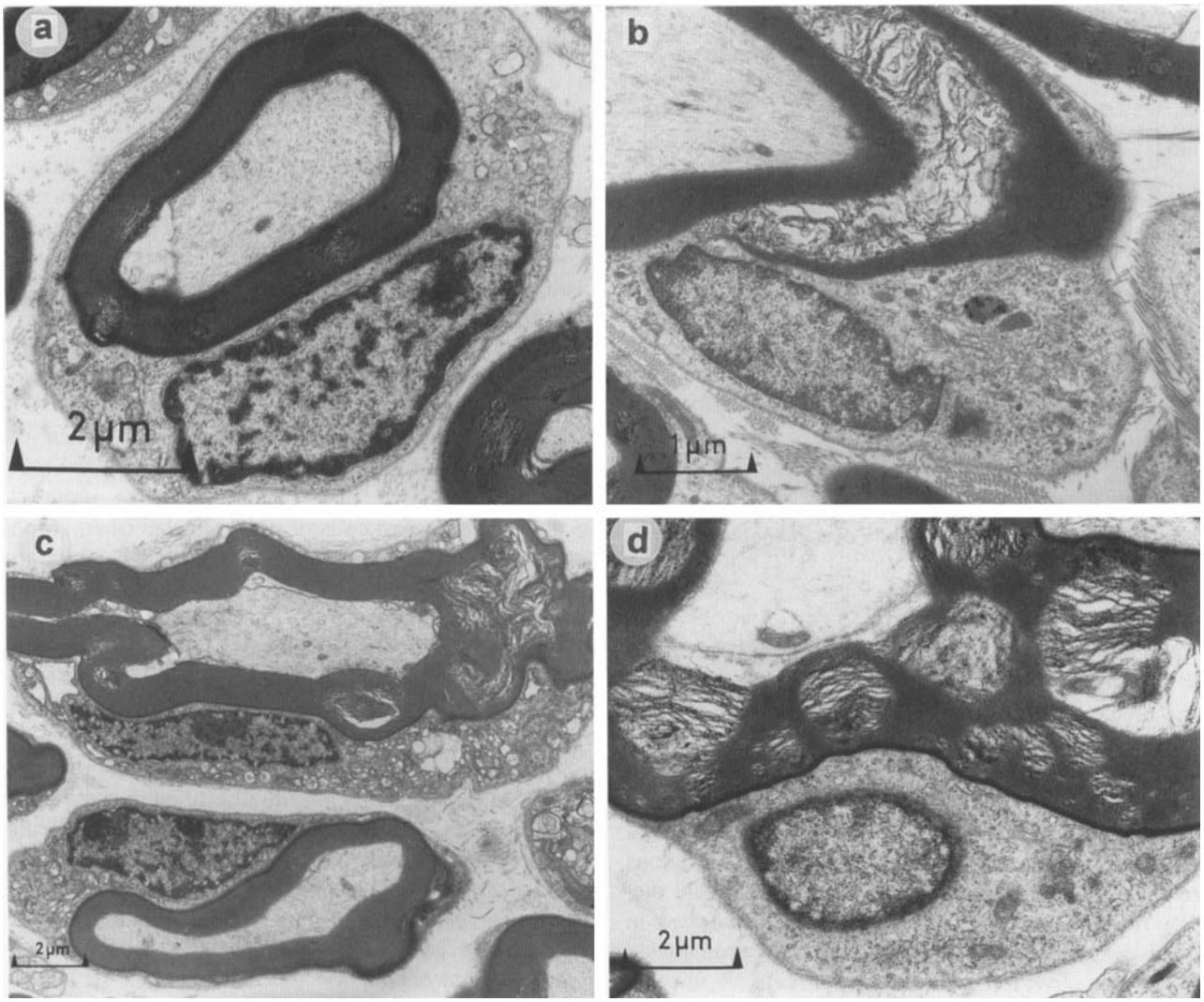

Fig. 1. Schwann cells of myelinated fibers. a: Control. Nucleus, cytoplasmic organelles, and myelin sheath are well-preserved. b: Cell of a nerve fiber of an animal in group III (total VCR dose, 24 μg). Heterochromatin is peripherally located, and damage to myelin sheath is evident. c: Two cells of a nerve fiber of an animal in group IV (total VCR dose, 96 μg). Upper cell shows marked damage on myelin sheath, whereas in lower cell, myelin sheath is fairly well-preserved. d: Portion of a cell of an animal in group V (total VCR dose, 400 μg). Severe damage to both nucleus and myelin sheath is seen.

RESULTS

The mice in group V, injected with the indicated dose, all died after 4 days or less. The blood counts showed no change in mice of groups II and III; a 37% decrease only of the platelet count was noted in group IV, whereas both white cell and platelet counts decreased by 86% and 63%, respectively, in group V. Hemoglobin and hematocrit levels remained unchanged in all experimental groups.

Electron Microscopy

Schwann cells of myelinated fibers. Control cells (Fig. 1a) showed the typical ultrastructure. The nucleus was oval or slightly invaginated in the vicinity of the myelin sheath, and contained peripherally-located heterochromatin and a round nucleolus. The cytoplasm contained a few mitochondria and short channels of endoplasmic reticulum. The myelin sheath was well-preserved, and the axons contained microtubules and microfilaments.

The cells of the animals of the second group (total VCR dose, 8 μg) did not show alterations of the nucleus and the cytoplasm. The myelin sheath and the axons appeared intact. Cells of the mice in the third group (total dose, 24 μg) (Fig. 1b) showed alterations of the nucleus consisting of an almost complete disappearance

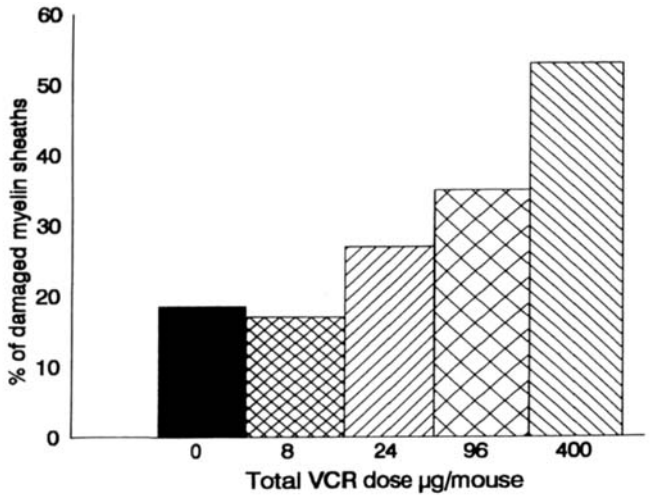

Fig. 2. Effect of escalating doses of VCR on ultrastructure of myelin sheaths in Schwann cells of myelinated fibers.

of the heterochromatin. The nucleolus was well preserved. The myelin sheath appeared swollen and disorganized. The damage was not observed in all cells: 27% showed alterations, and the rest appeared intact. The cells of animals in the fourth group (total dose, 96 μg) showed changes similar to those described in group III, except that the myelin sheath appeared damaged in a greater percentage of cells (35%) (Fig. 1c). The axons showed an increase in number of mitochondria and disrupted microtubules and microfilaments. Intact Schwann cells also were seen in this group. The most pronounced damage was observed in the Schwann cells of mice in the fifth group. Here again, the damaged organelles were the myelin sheaths (Fig. 1d), 53% of which showed numerous onion-like myelin figures. The axons were also impaired; the filaments, mitochondria, and microtubules were missing. A graphic representation of the percentage of damaged myelin sheaths is given in Figure 2.

Schwann cells of unmyelinated fibers. Cells of the control group showed a well-preserved nucleus and nucleolus, with peripherally located heterochromatin (Fig. 3a). Several axons were seen in a single cell, and the mesaxons were well-preserved. Some of them contained small, round mitochondria. Similar findings were observed in the cells of animals of the second group. In group III, in contrast to cells of the myelinated fibers, those of the unmyelinated fibers appeared well preserved (Fig. 3b). In cells of the fourth group, axonal damage in the form of loss of microfilaments and microtubules was observed (Fig. 3c). In addition, the cells showed a marked tendency to vacuolization. These findings were more pronounced in the fifth group, and were observed in a greater number of cells; however, the damage was less extensive compared to that observed in the myelinated cells of the mice in the same group (Fig. 3d).

DISCUSSION

Results of the present study indicate that VCR induces dose-dependent ultrastructural damage to Schwann cells of the mouse sciatic nerve. While mice receiving a total dose of up to 8 μg/mouse did not have pathological changes, those treated with higher doses, from 24 μg up, showed progressive damage to Schwann cells. Although all organelles were involved, the myelin sheath was the most affected. The higher the VCR dose, the more pronounced the damage to the individual cell, and the greater number of cells affected. When the damage induced by equal doses was compared, Schwann cells of myelinated fibers appeared to be more affected than Schwann cells of unmyelinated fibers. In the latter, alterations were observed beginning with total VCR dose of 96 μg per mouse, with more damage to the axons.

VCR-induced loss of myelin and axonal degeneration of peripheral nerves by VCR was reported by Moress et al. [7]. Shelanski and Wisniewski [8] described 3 patients with neurofibrillatory degeneration following Vinca alkaloid therapy. Similar findings were observed by the same authors in rabbits following intrathecal administration of the drugs. McLeod and Penny [9] performed a fascicular biopsy of the sural nerve of a patient treated with VCR and found mainly axonal degeneration, with demyelination to a lesser extent. According to these authors, axonal degeneration could explain the lack of major impairment in nerve conduction observed in their patients, a finding more indicative of segmented demyelination. Gottchalk and Dyck [1] reported ballooning and degeneration of myelinated fibers, as well as clumping of myelin in neurilemmal tubules in the peripheral nerves of mice treated with VCR. This was supported by findings in human nerves following treatment with VCR. In an experimental model using neurons in the cerebral ganglia of the snail *Lymnaea stagnalis,* Muller et al. [10] found that after treatment with VCR there appeared paracrystalline inclusions, chromatin patches, loss of arrangement of endoplasmic reticulum, and swelling of their cisternae. These findings were less prominent after treatment with other vinca alkaloids, such as vindesine and vinblastine. According to Thant et al. [11], when VCR is given in combination with VP-16, its toxic effect is potentiated, resulting in peripheral neuropathy with persistent wrist or foot drop and inability to walk. Electron microscopy examination of the peripheral nerves of these patients showed distinct changes in the myelin lamellae with presence of electron-dense material within the concentric layers, especially those adjacent to the axons. The mitochondria appeared swollen. The results of our study,

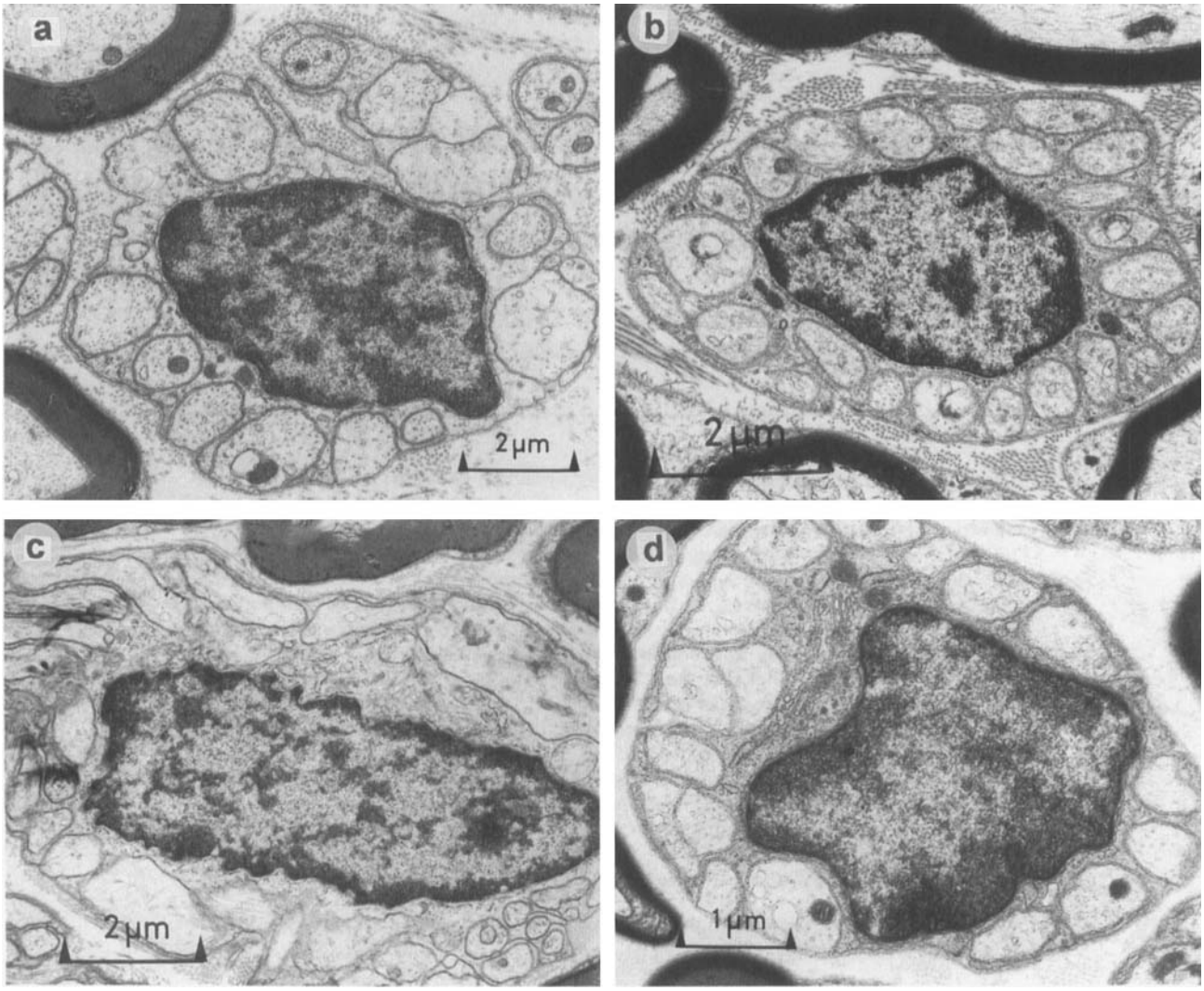

Fig. 3. Schwann cells of unmyelinated fibers. a: Control cell showing well-preserved nucleus and axons. b: Cell in nerve fiber of a mouse in group II (total VCR dose, 24 μg). Ultrastructure appears undamaged. c: Cell of a fiber in animal in group IV (total VCR dose, 96 μg). Axons are damaged and show a tendency for vacuolization. d: Cell in a fiber of an animal in group V (total VCR dose, 400 μg). Damage to nucleus and axons is evident.

showing that the most prominent damage of VCR treatment occurs in the myelin sheath, are in accordance with their findings.

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